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論文内容の要旨

The quaternary structural change of human hemoglobin A (Hb A) was studied by resonance Raman spectroscopy. The quaternary structural change of Hb A occurs upon ligand (oxygen, carbon monoxide (CO) and nitric oxide (NO)) binding to hemes and has a close correlation with oxygen affinity and cooperativity. It is known that no ligand-bound form of Hb A (deoxyHb A) adopts T (tense) structure with the low affinity extreme and that fully ligand-bound form of Hb A (COHb A) adopts R (relaxed) structure with the high affinity extreme. The most important problem to be answered for the quaternary structural change of Hb is when and how it occurs. However, generally it is difficult to detect a partially ligand-bound form of Hb A in a solution condition, because most of Hb A molecules are present in either no ligand-bound form or fully ligand-bound form. Therefore, in this study were selected the following Hbs in which a partially ligand-bound form can be stabilized in solution conditions: NOHb, Ni-Fe hybrid Hb, and Hb M Boston.

In Part I the results from ultraviolet resonance Raman (UVRR) studies on NOHb ($\alpha^{NO}\beta^{deoxy}$), Ni-Fe hybrid Hb ($\alpha^{Ni}\beta^{CO}$ and $\alpha^{CO}\beta^{Ni}$), and Hb M Boston ($\alpha^{Mmet}\beta^{CO}$) will be discussed. NOHb has the property that NO binds heme more strongly than CO. As the dissociation rate of NO is quite different between the α-heme and β-heme, it is possible to prepare a stable intermediate in which NO is bound only to α-heme. In this case, a condition of the Fe-His bond can be controlled by pH or addition of IHP: the heme can be made five- or six-coordinate state. Therefore the author can investigate the effect of the Fe-His bond of α-heme on the quaternary structural change of tetramer. Ni-Fe hybrid Hb has the property that CO does not bind to the Ni-heme. In this case, the author can investigate the quaternary structure of a half ligand-bound form. Hb M Boston has the property that CO does not bind to ferric α-abnormal chain. Hb M Boston is not exactly the same as Hb A owing to the difference in a distal residue of α-chain. Recently it is reported that Hb M Boston has cooperativity at high pH (pH = 9). This suggests that Hb M Boston may induce a quaternary structural change. The quaternary structure of Hb M Boston in a partially ligand-bound form can be studied. The largest structural differences between the T and R structures, revealed by X-ray crystallographic analysis, are located in the α1-β2 subunit interface. In this study an amount of the T and R structures in the intermediate states of quaternary structural change was estimated from UVRR spectral changes of the bands of tyrosine (Tyr) and tryptophan (Trp) residues. The quaternary structural change from T structure (deoxyHb A) to R structure (COHb A) results in the lower frequency shifts of Y8a and Y9a bands of tyrosine and intensity reduction of W3, W16, W18 bands of tryptophan.

The results from the measurements of the three Hbs are as follows. The quaternary structures of partially ligand-bound forms in NOHb ($\alpha^{NO}\beta^{deoxy}$), Ni-Fe hybrid Hb ($\alpha^{Ni}\beta^{CO}$) and ($\alpha^{CO}\beta^{Ni}$), and Hb M Boston ($\alpha^{Mmet}\beta^{CO}$) depend on pH and the absence or presence of IHP(inositol-hexakis-phosphate), and cannot be described by superimposition of only T and R structures which are limiting structures. Generally the ligand (CO not NO) binding to α-heme causes both lower frequency shifts of the special bands of tyrosine and intensity reduction of the special bands of tryptophan, but the ligand binding to β heme causes only the intensity reduction of the special bands of tryptophan, although the ligand binding to either α□, or β□heme at lower pH (pH 6.3~6.7) in the presence of IHP apparently causes no spectral change. This suggests that the roles of α-heme and β-heme (their Fe-His bonds) in the quaternary structural change are different. Binding of NO and CO to α-heme yields clear difference between the two ligands. Although the special bands of both tyrosine and tryptophan changed in the case of CO, the special bands of neither tyrosine nor tryptophan changed by NO even at pH 8.8 in the absence of IHP. This suggests that the difference in coordination ability between CO and NO influences the proximal His-Fe bond in α-heme, which reflects the large difference in the quaternary structural change.

On the other hand, the ligand binding to β-heme can be discussed from another views when $\alpha^{Ni}\beta^{CO}$ and Hb M Boston ($\alpha^{Mmet}\beta^{CO}$) are compared with $\alpha^{NO}\beta^{NO}$, because $\alpha^{NO}\beta^{deoxy}$ showed T structure at even higher pH (pH = 8.8) in the absence of IHP. But $\alpha^{NO}\beta^{NO}$ showed the R characteristics including both the lower frequency shifts of special bands of tyrosine and intensity reduction of special bands of tryptophan. It is different from the case of $\alpha^{Ni}\beta^{CO}$ (or $\alpha^{Mmet}\beta^{CO}$) that the NO (or CO) binding to β-heme in $\alpha^{NO}\beta^{deoxy}$ causes the lower frequency shifts of the special bands of tyrosine at lower pH and in the presence of IHP. The most important difference between $\alpha^{Ni}\beta^{CO}$ (or $\alpha^{Mmet}\beta^{CO}$) and $\alpha^{NO}\beta^{NO}$ is whether the sixth coordination site of α-heme is occupied by a ligand (NO) or not. This suggests that the quaternary structural change caused by the CO (NO) binding to β-heme depends on the coordination state in α-heme. The network involving the distal histidine such as Fe-NO---His in α-heme may also have a close connection with the change of tyrosine.

In conclusion of Part I, the change of tryptophan and tyrosine upon the quaternary structural change due to ligand (CO) binding to α-heme or β-heme can be summarized in the following way. CO binding to α-heme causes changes of both tryptophan and tyrosine and the changes do not depend on the state of β-heme. On the other hand, CO binding to β-heme causes a change of tryptophan only, but the change of tyrosine strongly depends on the state of α-heme. Thus, CO binding to α-heme seems to induce the quaternary structural change more strongly than that to β-heme.

In Part II, the relation between the function and structure of Hb which has very low affinity and apparently no cooperativity is treated. This type of Hb can be prepared under low pH in the presence of strong allosteric effector such as bezafibrate (BZF). Generally it has been considered that binding of ligands to Hb causes a quaternary structural change. However it is reported that ligand binding of Hb occurs with no cooperativity but that judging from the ^{1}H NMR signal, the quaternary structural change takes place. To investigate the relation between quaternary structure and cooperativity, this type of Hb is examined with resonance Raman spectroscopy.

The quaternary structural change upon ligand (CO) binding was also observed by resonance Raman spectroscopy for Hb which has very low affinity and apparently no cooperativity due to the strong allosteric effector. The R structure in the presence of the strong allosteric effector was not spectrally different from the R structure of normal HbA. The effects of strong allosteric effector appeared in a rate of structural relaxation after CO photodissociation, which is usually of microsecond order. In the presence of allosteric effector, the structural change from R-structure to T-structure becomes faster. The Fe-His stretching frequency at 13 μs after CO photodissociation at pH 6.4 in the presence of IHP and BZF was lower by 5 cm^{-1} than that observed in the absence of the effectors, for which the number of CO molecules remaining on hemes was estimated to be 2.8. When the number of CO molecules bound to hemes was changed, the degree of the quaternary structural change from R-structure to T-structure was also changed. At pH 6.4 in the presence of IHP and BZF the quaternary structural change from R-structure to T-structure has finished at 4 μs after CO photodissociation, even if the number of CO molecules remaining on hemes is 3.5. However, at pH 8.8 in the absence of the effectors the quaternary structural change from R-structure to T-structure has not been completed at 13 μs, even if the number of CO molecules bound to hemes is 2.8. This suggests that the quaternary structural change from R-structure to T-structure occurs between R_4 and T_3 at pH 6.4 in the presence of IHP and BZF, and that at pH 8.8 in the absence of the effectors quaternary structural change from R-structure to T-structure has not been completed yet in 13 μs after CO photodissociation or that T structure cannot be maintained when the number of CO molecules bound to hemes is 2.8. This indicates that mixed allosteric effector, IHP and BZF, shifts the transition point of the quaternary structure from $T_2 \rightarrow R_3$ to $T_3 \rightarrow R_4$.

論文の審査結果の要旨

本論文は約150ページの英文で書かれ，第２～第５章の各章が第一著者として外国の一流誌に印刷または投稿された論文に相当する。それ以外に本主題に関して４報の論文を発表しているが，それは学位論文には含まれていない。その他関連テーマに関して22報の論文が既に印刷されており，更にそれとは全く異なる分野の物理化学の論文が修士課程の成果として発表されている。したがって論文博士としてのバックグランドは十分に備えている。

本論文の第１章はヘモグロビンという分子の性質，すなわち協同的酸素結合をする事がその生理機能につながっている特質と，分子科学として何を解明すべきかを説明し，本研究の位置づけを分かりやすく説明している。また，本研究で採用する共鳴ラマン分光法の特色，とくに紫外共鳴ラマン
分光法の与える情報が解明すべき問題に対する鍵情報を与える可能性について説明している。その後の部分はPart I と II の２つに分けられ，Part I ではリガンド（O_2, CO, NO）結合過程の中間体，すなわちリガンドが半分（２個）結合した状態のスペクトルを決める方法とその結果が述べられ，
Part II では強いアロステリックエフェクターの存在下で見かけ上酸素結合の協同性が見えない状態でも，４次構造変化が起こる事の分光学的証拠とその説明が与えられている。

Part I には３つの章が含まれ，それぞれ別種の分子の定常状態紫外共鳴ラマンスペクトルの測定とその解釈が記述されている。分子種は異なるが問題にしている事は共通である。ヘモグロビンはαサブユニットが２個，βサブユニットが２個のテトラマー分子であるが，酸素結合に協同性があるためリガンド結合の中間段階を実験的に調べにくい。したがって，２状態モデルや連続変化モデルの実験的検証をしにくい。そこで，２個しかリガンドが結合できないヘモグロビンを３つの方法で用意し，リガンドがα或いはβサブユニットにのみ２個結合した状態の紫外共鳴ラマンスペクトルを調べた。

第２章はNOがαヘムに高い親和性で結合する事に注目し，pHを変える事によってαヘムのFe-His（ヒスチジン）結合が切れた状態と切れていない状態でαとβの界面接触構造がどう違うかを調べたものである。第３章は金属混合ヘモグロビンで，αにNi，βにFe，或いはその逆の組合せで，Niにはリガンドが結合し得ないので，Feにリガンドが２個結合した時と結合してないときとでαとβの界面がどう違うかを明らかにし，αとβは等価ではない事，トリプトファンとチロシンの変化は同期しない事等，新しい発見がいくつか述べられている。第４章はヒトミュータントヘモグロビンでFe^{2+}とFe^{3+}ヘムの組み合せになるものを選び，上記の問題を議論した。この場合はFe^{3+}ヘムにはリガンドが結合しない。

Part II は第５章のみであるが，普通の方法ではつくり出しにくいリガンド結合中間体をレーザー光によるリガンドの光解離でつくり出し，例えばリガンドが３個結合した状態を過渡状態として得て，リガンド結合数と４次構造との関係を議論している。TからRへの４次構造変化が普通のヒトヘモグロビンでは結合した酸素分子数が２個と３個の間で起こるが，IHPとBZFという２種のエフェクターの共存下では３個と４個の間で起こる事を初めて見つけた。それ故酸素結合に協同性は見られないが，４次構造変化は起こっている。これはラマン分光法ならでは得難い情報であり，その説明はヘモグロビン研究の専門家に

も新しいユニークな面を含んでいる。

このように本論文はヘモグロビンの４次構造変化を紫外共鳴ラマン法で調べた研究として国際的に最先端のものであり，質，量共に論文博士としての基準を十分に満たすものであるという事に全審査員の意見が一致した。

口述試験は約１時間の発表，約１時間の質疑応答として実施した。　申請者は学位論文の内容を１時間でわかりやすく説明した。質疑応答では，ヘモグロビンのＴ構造からＲ構造への転移の過程における構造変化の具体的な描像などの困難な質問にもよく答えた。また本論文でとった方法論に対する可能性と限界や，スペクトル解釈の問題点，誤差等についても，客観的に適切に理解して研究をすすめていることがわかった。また研究者としての基礎学力としても十分であることが認められた。論文は英語で記されており，また海外での学会発表の経験もあることから，語学力は十分と判断した。従って，口述試験は合格という事で委員全員の意見が一致した。公開発表では規定の時間で論文内容を的確に説明し，質問に対して正しく応答した。